EFFECTS OF DENTAL FLUOROSIS AND SALIVARY CONTAMINATION ON MICROLEAKAGE OF FOUR DIFFERENT RESTORATIVE MATERIALS IN PRIMARY MOLARS

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SUMMARY: In this *in vitro* study we evaluated the microleakage (entrance of microorganisms and toxins between the restoration and cavity walls of teeth) of Class V (kidney-shaped) cavities restored with four different types of restorative materials: a reinforced glass-ionomer cement, a resin-modified glass-ionomer cement, a compomer, and an ormocer. The effects of dental fluorosis and salivary contamination on microleakage of these materials were compared in primary molar teeth. Ninety-six Class V cavities were prepared on the buccal and lingual/palatinal surfaces of 48 non-carious human primary molars, half of which had fluorosis TFI (Thystrup and Fejerskov index) scores of 4. In both the fluorosed and control group, teeth were randomly assigned to two subgroups of 12. The first twelve teeth in each group were salivary contaminated prior to restoration. All restorations were placed strictly according to the instructions of the manufacturers. The samples were immersed in methylene blue, embedded in acrylic resin, sectioned and analyzed using stereomicroscopy. Statistically significant difference between the fluorosis and nonfluorosis groups was observed only for the gingival margins (p=0.021). Of the restorative materials tested, the resin-modified glass-ionomer cement was found to be more effective than the other materials in reducing microleakage both in the presence of fluorosis and salivary contamination.

Keywords: Class V cavities; Dental restoratives; Fluorosed teeth; Microleakage; Primary molars; Salivary contamination.

INTRODUCTION

For many decades, silver amalgam has been the standard restorative material in pediatric dentistry. However, the detrimental environmental effects of mercury, debates on possible health effects of amalgam, and the growing interest of patients and parents in enamel-colored restorations have resulted in a considerable reduction in the use of amalgam in dentistry. The most frequently used alternatives to amalgam for restoring primary teeth have been glass-ionomer cements (GICs), resin modified glass-ionomer cements (RMGICs), compomers, and resin composites (RCs).

Microleakage is a common problem in restorative dentistry and is defined as the leakage of microorganisms and toxins into teeth between the restoration and cavity walls of teeth.

In recent years, there has been growing evidence that the prevalence of dental fluorosis is increasing in both optimally and negligibly fluoridated communities.[1,2] Although, there have been many investigations of the adhesion and microleakage of restorative materials in nonfluorotic teeth, there appears to be limited information available on microleakage and bonding to the tooth structure when fluorosis is present, especially for the primary teeth.[3]

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The aim of this *in vitro* study was to compare the level of microleakage of four different restorative materials: a reinforced glass-ionomer cement (GIC), a resin-modified glass-ionomer cement (RMGIC), a compomer, and an ormocer, in Class V (kidney-shaped) restorations of primary molars with dental fluorosis. In addition, the effect of salivary contamination on microleakage of these restoratives was evaluated.

MATERIALS AND METHODS

Freshly extracted 48 noncarious primary molar teeth with a maximum of 1/2 root resorption were stored at 4ºC in 10% formalin solution for one month for use in this study. These molars were visually free of cracks and restorations, and half of them had fluorosed enamel. Because of its sensitivity and reproducibility, the Thylstrup and Fejerskov index (TFI)[4] was used for determining the severity of fluorosis of the extracted primary molars, and only teeth with TFI scores of 4 were selected and used for the study.

In both the fluorosis and control group, teeth were randomly assigned to two subgroups, and the first 12 teeth in each group were salivary contaminated prior to restoration. In total, ninety-six standardized rectangular Class V preparations were drilled on both buccal and lingual surfaces of 48 teeth, all by one dentist, using a high speed hand drill with air and water spray and a cylindrical diamond bur (Diatech Dental AG, CH-9435 Heerbrugg, Switzerland). The cavities were prepared as 3-mm mesio-distally, 2-mm occluso-gingivally with a 2-mm depth through the dentin layer, and all the cavity margins were located in the enamel. A calcium hydroxide lining (Life, Kerr, Romulus, MI, USA) was applied on the pulpal cavity walls of the preparations.

A reinforced glass-ionomer cement with the trade name Ketac Silver Aplicap (ESPE, Seefeld, Germany), a resin-modified glass-ionomer cement which has the trade name Vitremer (3M, St Paul, MN, USA), a compomer with the trade name Dyract AP (Caulk, Milford, DE), and an ormocer which has the trade name Admira (Voco, Cuxhaven, Germany) were the restorative materials used to restore the cavities.

Fresh nonstimulated human saliva was collected from five healthy, volunteer children who had not eaten or consumed any liquids for 30 minutes and pooled for immediate use.

All restorations were placed in strict accordance with instructions of the manufacturers for dentin conditioning, powder/liquid proportioning, and mixing.

After 24-hr storage in distilled water at 37ºC, the restored teeth were thermocycled for 100 cycles between two baths at 5±2ºC and 55±2ºC for 30 sec in each bath with intervals of 30 seconds between them. The specimens were then prepared for dye exposure. Subsequently, they were immersed in a buffered 2% solution of methylene blue for 24 hr at 37ºC and at the end of this period, removed from the dye and gently rinsed in order to remove excess dye. After the teeth were thoroughly washed with water, each tooth was subsequently embedded in orthodontic acrylic resin and sectioned longitudinally in a bucco-lingual direction through the center of each restoration with a water-cooled, low-speed diamond disc. The cut surfaces were examined at the occlusal and gingival margins using

Olympus SC 35 stereomicroscopy at ×20 magnification. Each section was independently scored by two evaluators. In the case of different readings recorded by the evaluators, mutual consensus was obtained by additional inspection, and the score with lowest reading was accepted. Two occlusal and two gingival microleakage scores of two halves of each tooth were recorded and linear microleakage of the restorations for both the occlusal and gingival margins was scored according to the scoring system used by Evancusky et al.[5]

The non-parametric data were analyzed by the Kruskal-Wallis analysis of variance test by ranks at a significance level of $p<0.05$. The Mann-Whitney U test was used to determine significant differences among the groups. Occlusal versus gingival microleakage scores were compared with the Wilcoxon matched-pairs signed-rank test. All statistical analysis procedures were performed by with SPSS statistical software release 10.0 standard version (SPSS Inc., 1999).

RESULTS

Tables 1 and 2 summarize the frequency of microleakage scores at the occlusal and gingival margins of fluorosis and nonfluorosis and salivary-contaminated and noncontaminated groups, respectively.

Table 1. Frequencies of microleakage scores (MLS) at the occlusal and gingival margins for the fluorosis and non-fluorosis groups

		Fluorosis group				Non-fluorosis group			
		Restorative material (see par. 3 of Materials and Methods for type)							
	MLS	Ketac Silver n (%)[a]	Vitremer n (%)	Dyract AP n (%)	Admira n (%)	Ketac Silver n (%)	Vitremer n (%)	Dyract AP n (%)	Admira n (%)
Occlusal	0	6 (6.3)	7 (7.3)	11 (11.5)	9 (9.4)	7 (7.3)	3 (3.1)	12 (12.5)	11 (11.5)
	1	3 (3.1)	13 (13.5)	5 (5.2)	3 (3.1)	2 (2.1)	5 (5.2)	5 (5.2)	5 (5.2)
	2	9 (9.4)	4 (4.2)	5 (5.2)	5 (5.2)	9 (9.4)	15 (15.6)	6 (6.3)	8 (8.3)
	3	6 (6.3)	-	3 (3.1)	7 (7.3)	6 (6.3)	1 (1)	1 (1)	-
	T[b]	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)
Gingival	0	5 (5.2)	10 (10.4)	4 (4.2)	4 (4.2)	3 (3.1)	10 (10.4)	8 (8.3)	9 (9.4)
	1	1 (1)	7 (7.3)	2 (2.1)	2 (2.1)	5 (5.2)	9 (9.4)	4 (4.2)	1 (1)
	2	1 (1)	4 (4.2)	5 (5.2)	8 (8.3)	3 (3.1)	4 (4.2)	7 (7.3)	7 (7.3)
	3	17 (17.7)	3 (3.1)	13 (13.5)	10 (10.4)	13 (13.5)	1 (1)	5 (5.2)	7 (7.3)
	T[b]	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)

[a] Percentages of microleakage scores are given in parenthesis.
[b] Total.

Higher leakage scores were observed for all restorative materials at the gingival margins than at the occlusal margins in the fluorosis group. In the nonfluorosis group, in contrast to the other restoratives, only the resin-modified glass-ionomer cement group showed higher occlusal than gingival leakage scores. Moreover, compomer restorations in the fluorosis group were observed to have higher gingival leakage scores than compomer restorations in the nonfluorosis group (Table 1).

Table 2. Frequencies of microleakage scores (MLS) at the occlusal and gingival margins for the salivary-contaminated and non-contaminated groups

		Salivary-contaminated group				Non-contaminated group			
		Restorative material (see par. 3 of Materials and Methods for type)							
	MLS	Ketac Silver n (%)[a]	Vitremer n (%)	Dyract AP n (%)	Admira n (%)	Ketac Silver n (%)	Vitremer n (%)	Dyract AP n (%)	Admira n (%)
Occlusal	0	4 (4.2)	5 (5.2)	4 (4.2)	3 (3.1)	9 (9.4)	5 (5.2)	19 (19.8)	17 (17.7)
	1	-	11 (11.5)	9 (9.4)	4 (4.2)	5 (5.2)	7 (7.3)	1 (1)	4 (4.2)
	2	10 (10.4)	8 (8.3)	7 (7.3)	10 (10.4)	8 (8.3)	11 (11.5)	4 (4.2)	3 (3.1)
	3	10 (10.4)	-	4 (4.2)	7 (7.3)	2 (2.1)	1 (1)	-	-
	T[b]	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)
Gingival	0	-	8 (8.3)	2 (2.1)	2 (2.1)	8 (8.3)	12 (12.5)	10 (10.4)	11 (11.5)
	1	5 (5.2)	10 (10.4)	1 (1)	-	1 (1)	6 (6.3)	5 (5.2)	3 (3.1)
	2	1 (1)	4 (4.2)	6 (6.3)	6 (6.3)	3 (3.1)	4 (4.2)	6 (6.3)	9 (9.4)
	3	18 (18.8)	2 (2.1)	15 (15.6)	16 (16.7)	12 (12.5)	2 (2.1)	3 (3.1)	1 (1)
	T[b]	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)	24 (25)

[a] Percentages of microleakage scores are given in parenthesis.
[b] Total.

In the salivary noncontaminated group, higher gingival leakage scores were observed than occlusal scores, except for the resin-modified glass-ionomer cement group, while in the salivary-contaminated group ormocer and reinforced glass-ionomer cement groups showed higher leakage scores than the resin-modified glass-ionomer cement group (Table 2).

The overall results are summarized in Tables 3 and 4. No statistically significant difference was found between the fluorosis and nonfluorosis groups for the microleakage scores at the occlusal margins (p>0.05), but the difference was statistically significant at the gingival margins (p=0.021). The restorative material groups which had statistically significant differences at the significance level of p<0.05 for both occlusal and gingival margins according to the Mann Whitney U test are shown in Table 3.

Table 3. Median leakage scores at the occlusal and gingival margins and significance levels for the fluorosis-non-fluorosis groups, salivary contaminated-non-contaminated groups, 'Ketac Silver-Vitremer', 'Dyract AP-Admira' groups and all restorative materials studied (see par. 3 of Materials and Methods for type)

	n	Median microleakage score (occlusal)	Median microleakage score (gingival)
Group I	96	1	1
Group II	96	1	2
Significance level (Mann Whitney U test, p=)		0.982	0.021
Group Ia, Group IIa	96	2	3
Group Ib, Group IIb	96	0	1
Significance level (Mann Whitney U test, p=)		0.001	0.001
Ketac Silver, Vitremer	96	2	1
Dyract AP, Admira	96	1	2
Significance level (Mann Whitney U test, p=)		0.009	0.316
Ketac Silver	48	2	3
Vitremer	48	1	1
Dyract AP	48	1	2
Admira	48	1	2
Significance level (Kruskal Wallis test, p=)		0.016	0.001
Significance level (Mann Whitney U test, p<0.05)		Ketac Silver-Dyract AP Ketac Silver-Vitremer Ketac Silver- Admira	Vitremer- Ketac Silver Vitremer- Admira Vitremer- Dyract AP Ketac Silver- Admira Ketac Silver- Dyract AP

There was a highly significant difference between salivary-contaminated and noncontaminated restoration groups both at occlusal and gingival margins (p<0.001). In the fluorosis/nonfluorosis groups, the restorative material which had statistically significant differences at the significance level of p<0.05 according to the Mann Whitney U test are shown at Table 4.

Table 4. Median leakage scores for the occlusal and gingival margins and significance levels due to fluorosis factor

	Group I			Group II		
	n	Occlusal (median)	Gingival (median)	n	Occlusal (median)	Gingival (median)
Salivary contaminated group	48	2	2	48	2	3
Non-contaminated group	48	0.5	1	48	0	1
Significance level (Mann Whitney U test, p=)		0.004	0.003		0.001	0.001
Ketac Silver, Vitremer	48	2	1	48	1	2
Dyract AP, Admira	48	1	2	48	1	2
Significance level (Mann Whitney U test, p=)		0.001	0.864		0.709	0.141
Ketac Silver	24	2	3	24	2	3
Vitremer	24	2	1	24	1	1
Dyract AP	24	0.5	1.5	24	1	3
Admira	24	1	2	24	1.5	2
Significance level (Kruskal Wallis test, p=)		0.006	0.004		0.094	0.001
Significance level (Mann Whitney U test, p<0.05)		V[a]-A[b] V-D[c] KS[d]-A KS-D	V-KS KS-D		V-KS	V-KS V-A V- D

V[a]: Vitremer (Resin-modified glass-ionomer cement)
A[b] : Admira (Ormocer)
D[c] : Dyract AP (Compomer)
KS[d]: Ketac Silver (Reinforced glass-ionomer cement)

DISCUSSION

Although much attention has been paid to the description of the microleakage and bond strengths of restorative materials in healthy permanent teeth, there is a lack of information about microleakage of restoratives in fluorosed teeth, especially in primary fluorosed teeth.

According to the results of this study, the resin-modified glass-ionomer cement showed the least microleakage scores in the fluorosis group. In these restorations improved adhesion is probably due to a physicochemical reaction with dentin and enamel due to the polar nature of the polyacrylate component and the formation of a hybrid layer from the hydrophylic hydroxyethylmethacrylate (HEMA).[6,7] The mechanism of adhesion is thought to be based on a dynamic ion-exchange process, in which the polyalkenoic acid component of the resin softens and infiltrates the hydroxyapatite structure of the tooth. It is hypothesized that calcium and phosphate ions are displaced out of the hydroxyapatite substrate to form an intermediate adsorption layer of calcium and aluminum phosphates and polyacrylates at the glass-ionomer-hydroxyapatite interface.[8,9]

The greater gingival than occlusal microleakage in the nonfluorosis group may be explained by the thicker enamel layer of the occlusal margins than gingival margins. It is possible that in resin-modified glass-ionomer cement restorations by the primer application, the pH of the dentin primer could modify the smear layer sufficiently to permit the tooth and the restorative material to come into intimate interfacial contact.[10]

From the clinical standpoint, contamination by saliva has always been a problem and can be especially difficult to control in the pediatric patient. Copious amounts of saliva, behavior management issues, very young patients, and rampant caries extending into cervical areas make isolation of dental surfaces difficult for placement of suitable restorations.

The results of this study indicate that salivary contamination adversely affects both occlusal and gingival microleakage when Class V cavities of primary molars are restored with the tested materials. This is in agreement with the results of Iovan et al.,[11] which reported that cervical microleakage could not be completely prevented in the presence of salivary contamination and that proper isolation should be mandatory. The significant increase in the microleakage scores in the presence of salivary contamination can possibly be explained by the reaction of dentin and saliva. The salivary proteins adsorb to the collagen meshwork and could prevent the penetration of the bonding agent.[5]

Ormocer and the reinforced glass-ionomer cement were the restoratives that were most affected by salivary contamination, with the resin-modified glass-ionomer cement showing the lowest microleakage scores in the salivary-contaminated group. The difference in performance may be explained by the chemical composition of the bonding agents and the amount of resin in the polymerized restorations. Compomer and ormocer have acetone-based bonding agents which are more affected by salivary proteins compared to the water-based bonding agents.[11]

Resin-modified glass-ionomer cement has 4.5–6% resin in the polymerized restoration, while compomer has more polymerizable resin than resin-modified glass-ionomer cement, but less than ormocer. Thus, in the salivary-contaminated group, microleakage scores for compomer were between these two materials.

According to the data obtained, microleakage cannot be prevented in the presence of salivary contamination. For Class V restorations of primary molars in the pediatric patient, especially both with fluorotic primary molars and with difficulties in controlling the salivary contamination, resin-modified glass-ionomer cement may be the restorative material of choice. However, these results should be supported by controlled clinical investigations.

Within the limits of this study, the following conclusions can be drawn:

1. Higher leakage scores occur at gingival margins for all restorative materials than at the occlusal margins in the fluorosis group. Apparently fluorosis adversely affects only the gingival microleakage.
2. Resin-modified glass-ionomer cement showed the least microleakage scores for both occlusal and gingival margins both in the fluorosis group and salivary-contaminated group and therefore can be recommended for Class V cavities in pediatric patients with fluorosed primary teeth.
3. Salivary contamination adversely affects both occlusal and gingival microleakage in Class V cavities of primary molars restored by all the tested restoratives.

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